

# The Role Of Hypoxia and Vascular Growth Factors in Experimental Atherosclerosis

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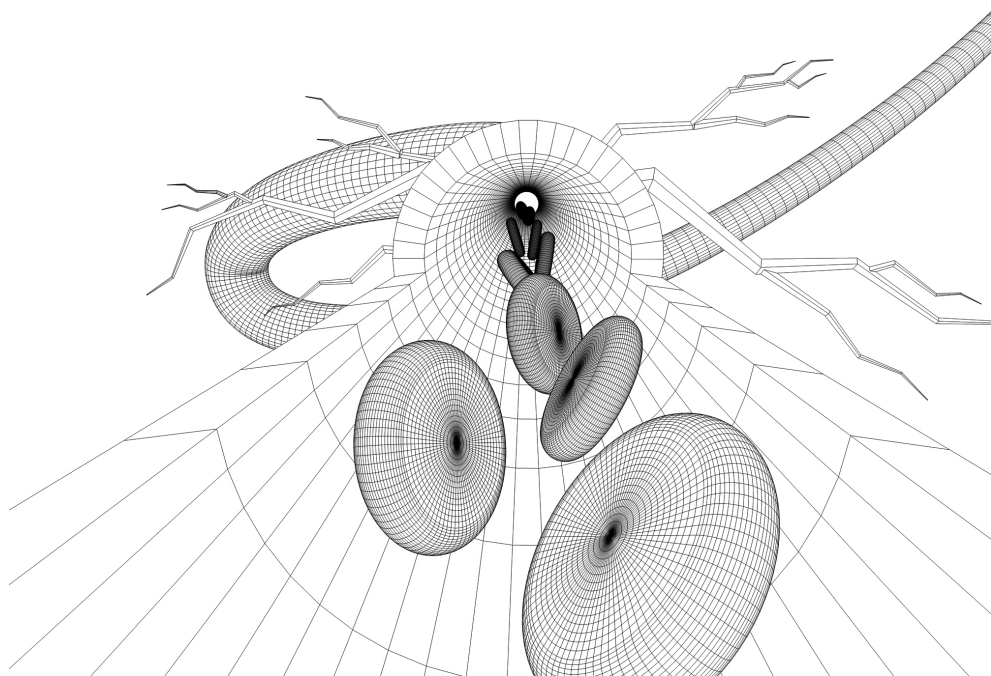
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# Chapter 9

## Valorization



## Atherosclerosis: a socio-economic burden

Valorization describes “the process of creating value from knowledge” in order to make knowledge suitable and/or available for social and/or economic use. In the opinion of the author the valorization section is of particular importance since it is the aim of the European Union (EU), in which the work was conducted (the Netherlands and Finland), to create a knowledge-based economy (1). The work presented in this thesis addresses atherosclerosis which is the underlying cause of most cardiovascular diseases and represents a major socio-economic burden. Cardiovascular diseases, such as myocardial infarction or stroke, annually account for 40% (1.9 million) of all deaths in the EU (2). Furthermore, as cardiovascular diseases are often not instantly lethal, patients may require life-long treatment and suffer from severe disabilities. In addition to the affliction of the patient, cardiovascular disease also has an impact on people in the patient’s close environment. Society as a whole is affected by cardiovascular disease as the related health care costs add up to annually 196€ billion in the EU (2).

Public funds are dedicated to two major themes: First, to the prevention of atherosclerosis and second, to its treatment. Reducing well established classical risk factors such as obesity, smoking, alcohol consumption, high blood pressure and high plasma LDL cholesterol helps to prevent atherosclerosis. However, not all cases can and will be prevented as unwillingness to adapt lifestyle, poor therapy compliance and genetic disorders such as familial hypercholesterolemia remain problematic. Therefore, exploring treatment options for existing and symptomatic atherosclerosis is a necessity. In 1987, statins, a drug primarily designed for cholesterol lowering, became commercially available and is since then the only frequently used treatment (3). Statins cannot cure cardiovascular disease as treatment reduces overall cardiovascular mortality with 12% and lowers the risk for major coronary and cerebrovascular events by 30% and 19% respectively (4). In addition long term statins use may cause side effects such as myopathy (5).

Thus, the current societal burden resulting from atherosclerosis raises demand for new innovative treatment strategies. This implies that important underlying experimental concepts to open new therapeutic opportunities have to be developed and need to become publicly available.

## Public availability of research results

The author’s interest in making the results of the publicly funded research available for society is depicted in the aim of publishing the obtained results in peer reviewed journals. This aim was accomplished in **chapter 2** and **chapter 5** as both are published in journals relevant for atherosclerosis research, in “Arteriosclerosis, Thrombosis, and Vascular Biology” and “Atherosclerosis” respectively. In addition to the already published chapters, **chapter 3** and **chapter 6** have been submitted for publication. The aim is to also publish the work presented in **chapter 4**, however, additional experiments are necessary to underline our preliminary findings. Publishing in peer reviewed journals does not only provide for knowledge dissemination, but also assures the scientific quality as experts in the field evaluate the submitted article prior to publication. The chosen journals are frequented by scientist, as well as medical personnel, making possible future translation of the research presented here more likely, as direct integration into the daily clinical

practice or commercial products is not applicable at the current state. The major limitation for a rapid translation is the use of mouse models, which do provide solid indications for basic mechanisms but unfortunately leave a gap between rodent and human atherosclerosis (6).

### **Generated novel insights with potential socio-economic value**

Clinical complications arising from atherosclerosis such as myocardial infarction or stroke are based on plaque rupture. Thus, delaying plaque formation or plaque stabilization of an atherosclerotic plaque in the performed animal experiments is defined by the author as finding with a potential value.

**Chapter 2** demonstrated how oxygen treatment successfully hampered atherosclerosis progression by reducing necrotic core formation in murine atherosclerosis. Furthermore, the underlying mechanism was partially unraveled which may represent a starting point for future therapeutic strategies. As oxygen treatment is also successful, but impractical in treating human atherosclerosis, the same underlying mechanisms found in the mouse model could be applied to humans. Mechanistically, it was found that hypoxia downregulated the efferocytosis receptor MerTK, resulting in accumulation of dead cells and necrotic core formation. After validation of the mechanism in humans, treatment aiming at upregulating MerTK receptor expression could be designed.

In **chapter 3** plaque stabilization, by enhancing plaque collagen and cap thickness after PHD2 inhibition in macrophages, was shown in an animal model. However, PHD2 inhibition simultaneously increased plaque size, demonstrating the risk for potential side effects of PHD2 inhibition. PHD inhibitors are currently investigated in clinical trials for the treatment of anemia in patients with chronic kidney disease (NCT02174731). These are neither isoform-specific, nor targeted to macrophages, but since PHD2 inhibition had an effect on experimental atherosclerosis (7), investigating its effects on atherosclerosis in the ongoing clinical trials could be considered. The effect of PHD inhibition could be ambivalent, thus, either the currently tested drug may be beneficial or harmful for atherosclerosis development. To obtain an isolated effect, isoform-specific, macrophage-targeted PHD inhibitors would need to be developed.

In **chapter 5** systemic Ang-2 inhibition was observed to decelerate plaque initiation likely via reducing plasma triglyceride levels. Reducing plasma triglycerides levels could also be beneficial for humans as these have a proven pro-atherogenic function. The anti-Ang-2 antibody used in chapter 2 is currently investigated in a clinical trial to treat unresectable stage III or stage IV melanoma (NCT02141542). Thus, the effect on triglyceride lowering in the animal model could be validated in the humans receiving the antibody. The frequent use of an antibody for reducing triglycerides is impractical for clinical practice as it is injected. General Ang-2 inhibition via small molecule inhibitors could overcome the practical concerns. However, Ang-2 is an important factor in angiogenesis and long term Ang-2 inhibition for triglyceride reduction may thus have unpredictable side effects. Besides, the presented results indicate a safe use of the Ang-2 antibody in cancer treatment with regard to atherosclerosis, but note that the data was generated based on an animal model.

**Chapter 6** shows the common regulation of several genes associated with endothelial hyper-permeability by VEGF-A and histamine. VEGF-A gene therapy bares the potential to treat ischemic diseases such as peripheral artery disease by the stimulation of

angiogenesis and subsequent revascularization of ischemic areas. A known side effect of VEGF-A gene therapy is the formation of edema as a consequence of its potential to evoke hyper-permeability. Our obtained results, the partial identification of the underlying pathways of VEGF-A and histamine induced hyper-permeability, might thus help to prevent edema formation in VEGF-A gene therapy thereby increasing the safety of this promising therapeutic tool. Amongst others MYCN and NR4A1 could be identified in the permeability induction cascade and their pharmacological inhibition by for instance existing CDK7 inhibitors or calcineurin inhibitors, respectively could be considered. However, additional experimental work is needed to verify the suggested approach for future clinical translation.

In conclusion, our findings are not applicable for direct translation into the clinical practice, but they provide a scientific basis for future developments in atherosclerosis research, and potentially even the development of new therapies. Furthermore, our results suggest to explore additional applications for existing therapeutic agents in atherosclerosis.

## References

1. European Council. Lisbon European Council Conclusions [Internet]. 2000 [cited 2016 Jan 15]. Available from: <http://www.consilium.europa.eu/en/workarea/downloadAsset.aspx?id=40802198157>
2. Nichols M, Townsend N, Luengo-Fernandez R, Leal J, Gray A, Scarborough P RM. European Cardiovascular Disease Statistics 2012. 2012.
3. Endo A. A historical perspective on the discovery of statins. *Proc Japan Acad Ser B*. 2010;86(5):484–93.
4. Brugts JJ, Yetgin T, Hoeks SE, Gotto AM, Shepherd J, Westendorp RGJ, et al. The benefits of statins in people without established cardiovascular disease but with cardiovascular risk factors: meta-analysis of randomised controlled trials. *BMJ*. 2009 Jun 30;338(jun30 1):b2376.
5. Kapur NK, Musunuru K. Clinical efficacy and safety of statins in managing cardiovascular risk. *Vasc Health Risk Manag*. 2008;4(2):341–53.
6. Libby P. Murine “Model” Monotheism: Figure. *Circ Res*. 2015 Nov 6;117(11):921–5.
7. Rahtu-Korpela L, Määttä J, Dimova EY, Hörkkö S, Gylling H, Walkinshaw G, et al. Hypoxia-Inducible Factor-Prolyl 4-Hydroxylase-2 Inhibition Protects Against Development of Atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2016 Feb 4;ATVBAHA.115.307136.

